Received: 22 January 2020 | Revised: 15 April 2020 | Accepted: 16 April 2020

DOI: 10.1111/bph.15089

RESEARCH PAPER

Inflammation induces developmentally regulated sumatriptan inhibition of spinal synaptic transmission

Bryony L. Winters | Hyo-Jin Jeong | Christopher W. Vaughan

Pain Management Research Institute and Kolling Institute of Medical Research, The University of Sydney at Royal North Shore Hospital, St. Leonards, New South Wales, Australia

Correspondence
Bryony L. Winters, Pain Management Research Institute and Kolling Institute of Medical Research, The University of Sydney at Royal North Shore Hospital, St. Leonards, New South Wales 2065, Australia.
Email: bryony.winters@sydney.edu.au

Funding information
NHMRC, Grant/Award Number: 1083569

Background and Purpose: While triptans are used to treat migraine, there is evidence that they also reduce inflammation-induced pain at the spinal level. The cellular mechanisms underlying this spinal enhancement are unknown. We examined whether inflammation alters sumatriptan modulation of synaptic transmission in the rat spinal dorsal horn.

Experimental Approach: Three to four days following intraplantar injection of complete Freund's adjuvant (CFA) or saline, whole cell recordings of evoked glutamatergic EPSCs were made from lumbar lamina I–II dorsal horn neurons in rat spinal slices

Key Results: In 2- to 3-week-old animals, sumatriptan reduced the amplitude of evoked EPSCs and this was greater in slices from CFA, compared to saline-injected rats. In CFA-injected animals, sumatriptan increased the paired pulse ratio of evoked EPSCs and reduced the rate of spontaneous miniature EPSCs. The 5-HT_{1B} and 5-HT_{1D} agonists CP9 3129 and PNU109291 both inhibited evoked EPSCs in CFA but not saline-injected rats. By contrast, the 5-HT_{1A} agonist R(+)-8-OH-DPAT inhibited evoked EPSCs in saline but not CFA-injected rats. In CFA-injected rats, the sumatriptan-induced inhibition of evoked EPSCs was reduced by the 5-HT_{1B} and 5-HT_{1D} antagonists NAS181 and BRL-15572. Intriguingly, the difference in sumatriptan inhibition between CFA and saline-injected animals was only observed in animals less than 4 weeks old.

Conclusion and Implications: These findings indicate that inflammation induces a developmentally regulated 5-$HT_{1B/1D}$ presynaptic inhibition of excitatory transmission into the rat superficial dorsal horn. Thus, triptans could potentially act as spinal analgesic agents for inflammatory pain in the juvenile setting.

1 | INTRODUCTION

Triptans, such as **sumatriptan**, are **5-HT_{1B}**, **5-HT_{1D}** and **5-HT_{1F}** receptor agonists that are commonly used for the treatment of migraine (Cameron et al., 2015; Humphrey et al., 1990; Monteith & Goadsby, 2011). Clinical and preclinical studies indicate that triptans produce their cranial antinociceptive effects by acting on 5-$HT_{1B/1D/1F}$ receptors in the periphery and 5-$HT_{1B/1D}$ receptors in

Abbreviations: ACSF, artificial CSF; AP5, DL-(−)-2-amino-5-phosphonopentanoic acid; BRL-15572, 3-[4-(4-chlorophenyl)piperazin-1-yl]-1,1-diphenyl-2-propanol hydrochloride; CFA, complete Freund's adjuvant; CNQX, 6-cyano-2,3-dihdroxy-7-nitro-quinoxaline; CP 93129, 1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5*H*-pyrrol[3,2-b]pyridin-5-one dihydrochloride; EGTA, ethylene glycol tetra-acetic acid; NMDG, *N*-methyl-D-glucamine; NAS181, (2R)-2-[[[3-(4-Morpholinylmethyl)-2H-1-benzopyran-8-yl]oxy]methyl]morpholine dimethanesulfonate; PNU109291, (*S*)-3,4-dihydro-1-[2-[4-(4-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-methyl-1*H*-2-benzopyran-6-carboxamide; QX-314, lidocaine N-ethyl bromide; R(+)-8-OH-DPAT, (2R)-(+)-8-hydroxy-2-(di-*N*-propylamino)tetralin hydrobromide; Sumatriptan, 3-[2-(dimethylamino)ethyl]-*N*-methyl-1*H*-indole-5-methanesulfonamide succinate; TTX, tetrodotoxin; WAY-100635, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide maleate.

Bryony L. Winters and Hyo-Jin Jeong contributed equally to this work.

cranial nociceptive afferents (Akerman et al., 2019; Akerman, Holland, Summ, Lasalandra, & Goadsby, 2012; Hoskin & Goadsby, 1998; Kayser, Aubel, Hamon, & Bourgoin, 2002; Nozaki, Moskowitz, & Boccalini, 1992). In addition to trigeminal targets, it has been demonstrated that 5-HT_{1B} and 5-HT_{1D} receptors are located in lumbar dorsal root ganglion neurons (Classey, Bartsch, & Goadsby, 2010; Pierce, Xie, Levine, & Peroutka, 1996; Potrebic, Ahn, Skinner, Fields, & Basbaum, 2003; Wotherspoon & Priestley, 2000). However, antinociceptive effects have not been consistently observed with spinally delivered triptans in studies on naïve animals (Alhaider & Wilcox, 1993; Ali, Wu, Kozlov, & Barasi, 1994; Connor et al., 1997; Jeong, Mitchell, & Vaughan, 2012; Nikai, Basbaum, & Ahn, 2008; Skingle, Birch, Leighton, & Humphrey, 1990; Xu, Qiu, & Han, 1994).

These functional and anatomical studies are reflected by in vivo electrophysiological studies, which have shown that triptans act via central 5-HT_{1B} and 5-HT_{1D} receptors to inhibit nociceptive transmission from pain sensitive intracranial structures within the trigeminal dorsal horn (Cumberbatch, Hill, & Hargreaves, 1998; Donaldson, Boers, Hoskin, Zagami, & Lambert, 2002; Goadsby & Hoskin, 1996; Levy, Jakubowski, & Burstein, 2004; Storer & Goadsby, 1997). Complementing this work, in vitro slice studies have shown that triptans presynaptically inhibit primary afferent evoked glutamatergic EPSCs in trigeminal dorsal horn neurons of naïve animals (Choi et al., 2012; Jennings, Ryan, & Christie, 2004; Travagli & Williams, 1996). By contrast, sumatriptan has little effect on primary afferent evoked EPSCs in spinal lumbar dorsal horn neurons of naïve animals (Garraway & Hochman, 2001; Hori, Endo, & Takahashi, 1996; Ito et al., 2000; Jeong et al., 2012; Li & Zhuo, 1998; Lu & Perl, 2007).

It is possible that the actions of different 5-HT_1 receptor subtypes might change in inflammatory pain states, where there is altered expression of 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors in dorsal root ganglia and spinal cord (Ahn & Basbaum, 2006; Wu et al., 2001; Zhang et al., 2002). In this regard, it has been demonstrated that lumbar intrathecal delivery of triptans reduces thermal hyperalgesia and mechanical allodynia in models of inflammation (Bingham et al., 2001; Nikai et al., 2008; Quinonez-Bastidas et al., 2018). This suggests that, unlike naïve animals, triptans may have spinal 5-$HT_{1B/1D}$ mediated analgesic actions in inflammatory pain states. In the present study, we examined whether inflammation alters the presynaptic actions of sumatriptan in the spinal cord slice preparation and the role of 5-$HT_{1B/1D}$ receptors in these effects.

What is already known

- 5-$HT_{1B/1D}$ agonists inhibit afferent inputs to the medullary but not the spinal dorsal horn.
- Triptans, which are 5-$HT_{1B/1D/1F}$ agonists, produce spinal analgesia in inflammatory pain states.

What does this study add

- Inflammation increases 5-HT_{1B} and 5-HT_{1D} presynaptic inhibition of afferent inputs to the spinal dorsal horn.
- This enhancement of sumatriptan presynaptic inhibition only occurs in young animals.

What is the clinical significance

- Triptans have potential as spinal analgesics for inflammatory pain in a juvenile setting.

2 | METHODS

Animal studies are reported in compliance with the ARRIVE guidelines (Kilkenny et al., 2010) and with the recommendations made by the *British Journal of Pharmacology*. Experiments were carried out on 1- to 8-week-old Sprague–Dawley male and female rats and followed the guidelines of the 'NH&MRC Code of Practice for the Care and Use of Animals in Research in Australia'. All experimental protocols were approved by the Royal North Shore Hospital Animal Ethics Committee (protocols 0912-020A, 1211-021A). Pregnant female rats were obtained from Animal Resources Centre (Canning Vale, Australia) and were housed in individually ventilated, translucent cages under a 12:12 h light/dark cycle, with environmental enrichment and free access to water and standard rat chow. When rat pups were born, each litter was housed together with its mother until P21, when they were weaned and housed in groups of three same-sex littermates per cage.

2.1 | Inflammation model

For the inflammatory pain model, animals were briefly anaesthetised with isoflurane (2–2.5% in O_2) and received an intraplantar injection of complete Freund's adjuvant (CFA, Sigma, Sydney, Australia) or saline into the plantar surface of the left hindpaw via a 30-gauge needle. The injected volume of saline/complete Freund's adjuvant was 0.45 μl per gram body weight (e.g., Walker, Meredith-Middleton, Cooke-Yarborough, & Fitzgerald, 2003). This equated to injection volumes of 10, 15, 20, 60, 80 and 120 μl in 1-, 2-, 3-, 4-, 5- and 6-week-old rats. Animals were used for electrophysiology experiments 3–4 days after intraplantar injection of saline/complete Freund's adjuvant

In some animals, the volume and mechanical paw withdrawal threshold of the operated hindpaw was measured prior to, and 3 days following intraplantar injection of complete Freund's adjuvant or saline. All behavioural measurements were carried under low level white light (<3 lux). Paw volume was measured using a plethysmometer (Ugo Basile). To reduce inter-animal variability, this was normalised to the percentage change in paw volume, compared

to the pre- complete Freund's adjuvant/saline value, and used for analysis. To assess mechanical allodynia, the mechanical paw withdrawal threshold (PWT) was measured with a series of von Frey hairs (range 0.16–6.0 g). Rats were placed in elevated Perspex enclosures with wire mesh bases and were given 20 min to acclimatise to the testing environment. Each von Frey hair was pressed perpendicularly against the hindpaw and held for approximately 2 s, four times at random locations over the plantar surface of the left hindpaw. The mechanical paw withdrawal threshold was measured using a simplified up-down paradigm (Bonin, Bories, & De Koninck, 2014) and raw values, before and after complete Freund's adjuvant/saline, were used for analysis.

2.2 | Electrophysiology

For electrophysiological experiments, animals were deeply anaesthetised with isoflurane and killed by decapitation. The spinal cord was rapidly removed, the dura incised and the spinal column removed and placed in ice cold sucrose cutting solution of composition (mM): Sucrose, 240; KCl, 3; NaH_2PO_4, 1.4; $MgCl_2$, 7; $CaCl_2$, 0.2; glucose, 11; $NaHCO_3$, 28; equilibrated with 95% O_2 and 5% CO_2. Transverse or parasagittal (300 μm) slices of the lumbar spinal cord (L4–6) were cut using a vibratome (VT12000S: Leica, Nussloch Germany). After cutting, slices were incubated in an NMDG recovery solution of composition (mM): NMDG, 93; KCl, 2.5; NaH_2PO_4, 1.2; $NaHCO_3$, 30, HEPES, 20; Glucose, 25; sodium ascorbate, 5; thiourea, 2; sodium pyruvate, 2; $MgCl_2$, 10; $CaCl_2$, 0.5; pH 7.4 with HCl, 300–310 $mOsm{\cdot}L^{-1}$ for 10 min at 34°C before being transferred to ACSF (Ting, Daigle, Chen, & Feng, 2014). Slices were recovered for 1 h at 34°C before being transferred to room temperature where they were maintained in a submerged chamber containing ACSF equilibrated with 95% O_2 and 5% CO_2, prior to recording.

For electrophysiological recordings, individual slices were transferred to a slice chamber (volume 0.5 ml) and continuously superfused (2.5 $ml{\cdot}min^{-1}$) with ACSF at 34°C using an inline temperature controller. Superficial dorsal horn neurons located throughout lamina I–II were visualised using Dodt-tube optics on an upright microscope (Olympus BX50, Olympus, Sydney, Australia). Whole cell voltage clamp recordings (holding potential −60 mV, liquid junction potential corrected) were made using Axopatch 200B/700B amplifiers (Molecular Devices, Sunnyvale, CA, USA). The internal solution contained (mM): CsCl 140, HEPES 10, EGTA 0.2, $MgCl_2$ 1, QX-314 3, MgATP 2 and NaGTP 0.3 (pH 7.3 and osmolality 280–285 $mOsmol{\cdot}L^{-1}$). Series resistance (<25 MΩ) was compensated by 80% and continuously monitored during experiments.

Electrically evoked EPSCs were elicited in neurons via a glass unipolar stimulating electrode placed on the dorsal roots adjacent to the dorsal root entry zone in the presence of the $GABA_A$ receptor channel blocker **picrotoxin** (100 μM), the glycine receptor antagonist **strychnine** (3 μM) and the NMDA receptor antagonist DL-**AP5** (25 μM). The stimulus intensity was set at 10× the threshold at which EPSCs were first detected (5–22 V, 0.1–0.2 ms duration). Evoked EPSCs were identified as monosynaptic when their initial peak displayed little jitter (variation in EPSC onset latency) and maintained amplitude during brief stimulation at 1 s^{-1}. Recording with a large polysynaptic component were excluded. Drug experiments on evoked EPSCs were subsequently performed by evoking paired EPSCs (interval = 70 ms) every 12 s. In separate experiments, spontaneous miniature EPSCs were obtained without electrical stimulation in the additional presence of tetrodotoxin (TTX, 500 nM). In these experiments, membrane conductance was assessed by applying a 20 ms long −5 mV step every 12 s. Stock solutions of all drugs were made in H_2O or DMSO and then diluted (1:3,000–10,000) to working concentrations in ACSF immediately before use and applied by superfusion to the slice chamber. 5-HT ligands were used at maximal concentrations, as described previously (Jeong et al., 2012).

Recordings were filtered (2 kHz low-pass filter) and sampled (10 kHz) for on-line and later off-line analysis using AxographX (Axograph Scientific, Australia). Evoked EPSC amplitude was measured as the difference between the peak of the EPSC and the average of the 1 ms period preceding the stimulus artefact. Miniature EPSCs above a pre-set threshold (3–4 SDs above baseline noise) were automatically detected by a sliding template algorithm to determine their amplitude, half-width and inter-event intervals.

2.3 | Data and analysis

The data and statistical analysis comply with the recommendations of the *British Journal of Pharmacology* on experimental design and analysis in pharmacology (Curtis et al., 2018). We aimed for a sample size of at least six neurons per experimental group, as we have previously observed effect sizes of at least 1.8 (for two-tailed tests with α, β errors of 0.05) for the presynaptic actions of 5-HT ligands in spinal cord slices from naïve animals (Jeong et al., 2012). In order to ensure this minimum sample size, we used six animals per experimental group in animals younger than P28 as we generally obtain one to two successful neuron recordings from different spinal slices per animal in this age group. In older animals, we used eight animals per experimental group because of reduced slice viability. The variability in neuron recordings was due to slice quality and the ability to obtain whole cell mode recordings with giga-ohm seals, plus stable EPSC parameters (see below). In addition, equal or near-equal numbers of neurons from male and female animals were used in all experimental groups to balance sexes.

The researchers performing electrophysiology experiments were blinded to the animal treatment group (saline, complete Freund's adjuvant) but not to the agonists/antagonists applied during experiments. For the experiments, baseline evoked or spontaneous miniature EPSCs were recorded for a minimum of 10 min in the presence of picrotoxin/strychnine/AP5, and where applicable, **TTX** and/or a 5-HT receptor antagonist. Between one and three different agonists (or agonist/antagonist combinations) were tested in each neuron, depending on cell viability. The experimenter sequentially rotated the order in which different drugs were examined in between

successive neuron recordings from different slices and animal. This reduced any potential bias resulting in the order of drug testing in each neuron. This also aided in spreading specific drug experiments across different animals. Thus, the majority of experiments testing specific drugs were made from neurons taken from different slices in different animals (approximately 90%). After the baseline period, serotonergic agonists were then applied for a period of 6 min. Neuron recordings were excluded from analysis if any EPSC parameter varied by more than 15% over the 6 min baseline period prior to agonist application. Drug effects on electrophysiological parameters were made by measuring at fixed time points: over the last 2 min prior to agonist application and at the end of agonist superfusion. Drug effects were measured by calculating the values of EPSC parameters in the presence of agonist as a percentage of the pre-agonist values to reduce variability in evoked EPSC amplitude between experiments.

Parametric statistical analysis was made using Prism (version 8, GraphPad Software, La Jolla, USA) and SPSS (IBM Corp., Armonk, USA). Comparison of the effect of inflammation on the percentage change in paw volume was made using two-way ANOVA (between subject factors = age, intraplantar treatment), and its effect on mechanical paw withdrawal threshold was made using three-way ANOVA (within subject factors = time; between subject factors = age, intraplantar treatment). For these experiments, post hoc comparisons were made using Bonferroni's adjustment. Statistical assessment of individual drug effects on EPSC parameters was made using one sample Student's paired *t*-tests. Comparisons of drug effects on EPSC parameters between neuron groups or antagonist treatment groups were made using Student's unpaired *t*-tests or one-way ANOVAs (between subject factors = neuron group or antagonist treatment). For these experiments, post hoc comparisons using Dunnett's or Tukey's adjustment where appropriate. Comparison of the effect of age on sumatriptan inhibition of evoked EPSCs between complete Freund's adjuvant- and saline-injected animals was made using two-way ANOVA (between subject factors = age, intraplantar treatment). For these experiments, post hoc comparisons between treatment groups were made using Bonferroni's adjustment. In addition, a comparison of the effect of age, treatment and sex was made using a three-way ANOVA. ANOVAs satisfied the criteria of homogeneity of variance (Brown–Forsythe test) and sphericity (Mauchly's test), and post hoc tests were conducted only when main effects or interactions were significant. Differences were considered significant when $P < 0.05$; all numerical data are expressed as mean ± SEM.

2.4 | Reagents

3-[4-(4-Chlorophenyl)piperazin-1-yl]-1,1-diphenyl-2-propanol hydrochloride (**BRL-15572**), 1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5*H*-pyrrol[3,2-b]pyridin-5-one dihydrochloride (**CP 93129**), (2R)-2-[[[3-(4-morpholinylmethyl)-2*H*-1-benzopyran-8-yl]oxy]methyl] morpholine dimethanesulfonate (**NAS181**), (*S*)-3,4-dihydro-1-[2-[4-(4-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-methyl-1*H*-2-benzopyran-6-carboxamide (**PNU109291**), (2R)-(+)-8-hydroxy-2-(di-*N*-propylamino) tetralin hydrobromide (**R(+)-8-OH-DPAT**) were obtained from Tocris Cookson (Bristol, UK); 6-cyano-7-nitro-quinoxaline-2,3-dione (**CNQX**), DL-(−)-2-amino-5-phosphonopentanoic acid (AP5), lidocaine N-ethyl bromide (QX-314) and tetrodotoxin citrate (TTX) from Abcam Biochemicals (Cambridge, UK); picrotoxin, strychnine hydrochloride, 3-[2-(dimethylamino)ethyl]-*N*-methyl-1*H*-indole-5-methanesulfonamide succinate (sumatriptan), *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-2-pyridinylcyclohexanecarboxamide maleate (**WAY-100635**) and other general reagents were from Sigma (Sydney, Australia). Stock solutions of neurochemicals were made in distilled water, or DMSO, aliquoted and then frozen. Individual aliquots of 5-HT ligands were then transferred to a fridge every 2–3 weeks and used for experiments.

2.5 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander, Christopoulos et al., 2019; Alexander, Mathie et al., 2019).

3 | RESULTS

3.1 | Inflammation increases sumatriptan inhibition of afferent evoked EPSCs in juvenile animals

We have previously shown that sumatriptan produces a modest 5-HT_{1A}-mediated inhibition of afferent evoked EPSCs in dorsal neurons from pre-weaned (P14–21) naïve rats (Jeong et al., 2012). We examined whether the effect of sumatriptan was altered in an inflammatory pain state by comparing the effect of sumatriptan on primary afferent evoked EPSCs in animals which had received an intraplantar injection of either complete Freund's adjuvant or saline prior to electrophysiological experiments. The percentage change in paw volume significantly varied with intraplantar treatment (Figure 1a). The percentage increase in paw volume of pre-weaned (P14–21) rats was significantly greater in those which received intraplantar complete Freund's adjuvant compared to those which received intraplantar saline, 3–4 days previously (Figure 1a). In addition, the change in mechanical paw withdrawal threshold significantly varied with intraplantar treatment (Figure 1b). In pre-weaned (P14–21) rats, there was a significant decrease in mechanical paw withdrawal threshold of complete Freund's adjuvant but not saline-injected animals (Figure 1b). Thus, intraplanar complete Freund's adjuvant but not saline-induced hindpaw inflammation and mechanical allodynia in pre-weaned animals.

In the presence of picrotoxin, strychnine and AP5 stimulation of the dorsal roots evoked synaptic currents in lamina I–II neurons from

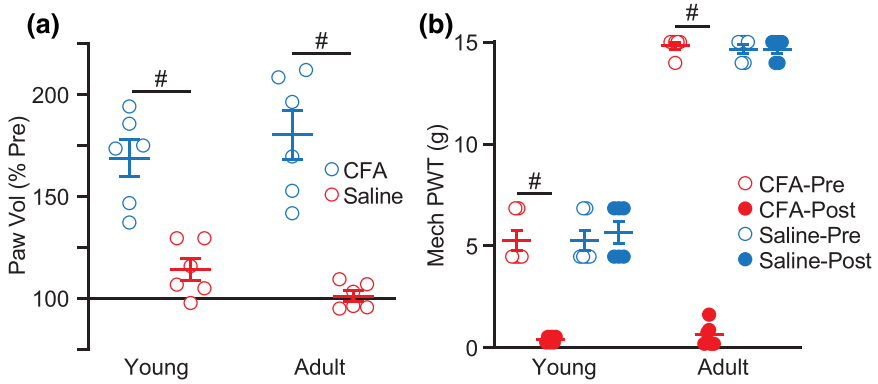

FIGURE 1 Intraplantar complete Freund's adjuvant (CFA) induces inflammation and mechanical allodynia on juvenile and adult rats. (a) Scatter plots of the percentage change in paw volume in juvenile (2 to 3 weeks old) and older (6 to 8 weeks old) rats 3–4 days after intraplantar injection of complete Freund's adjuvant CFA or saline. (b) Scatter plot of the mechanical paw withdrawal threshold (PWT) in juvenile and older rats prior to, and 3–4 days after intraplantar injection of CFA or saline. In (a) and (b) # denotes $P < 0.05$ for post hoc comparisons, $n = 6$ per group

both complete Freund's adjuvant- and saline-injected pre-weaned rats, which were abolished by the non-NMDA receptor antagonist CNQX (10 μM) (e.g. Figure 2a,b). In dorsal horn neurons ipsilateral to the complete Freund's adjuvant-injected hindpaw of these animals, superfusion of a maximal concentration of sumatriptan (3 μM) produced a significant decrease in the amplitude of evoked EPSCs which did not always reverse upon washout (Figure 2a,c). In these neurons, sumatriptan did not have an effect on membrane conductance (103 ± 5% of pre-sumatriptan, p > 0.05). Sumatriptan also produced a significant decrease in the amplitude of evoked EPSCs in dorsal horn neurons ipsilateral to saline-injection which reversed upon washout (Figure 2b,c). Furthermore, sumatriptan produced a significant decrease in the amplitude of evoked EPSCs in dorsal horn neurons contralateral to complete Freund's adjuvant-injection (Figure 2c). The

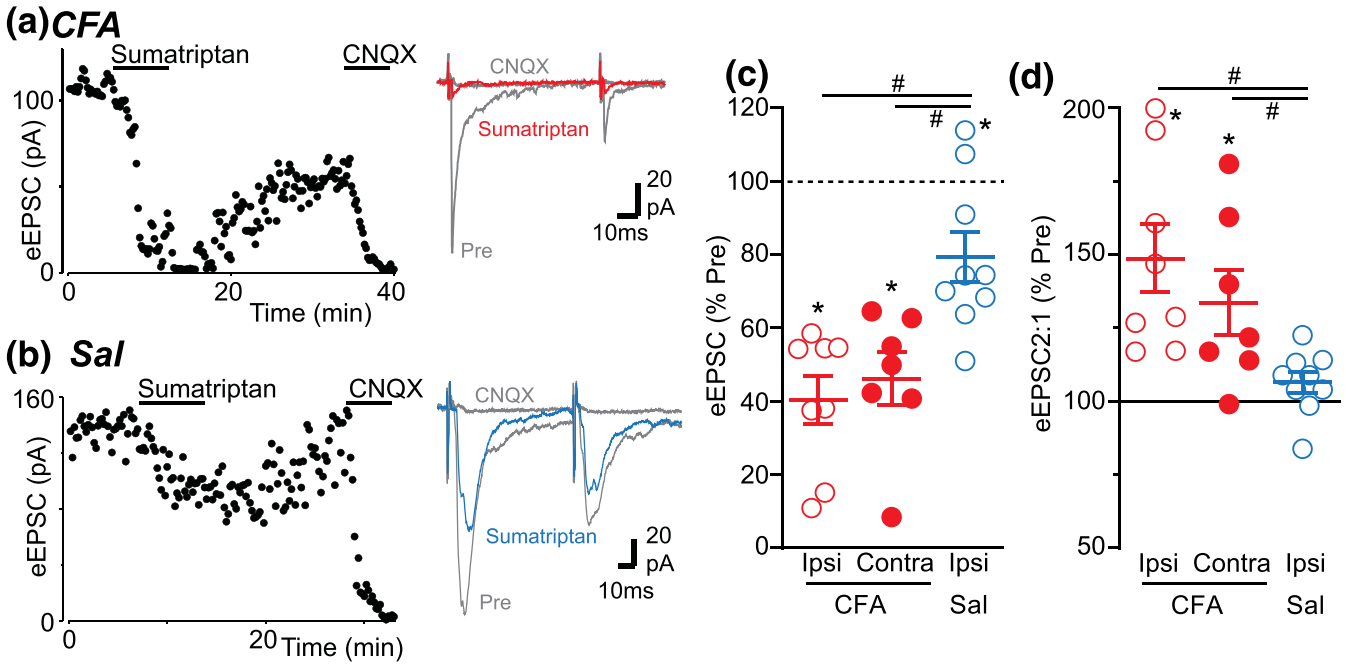

FIGURE 2 Sumatriptan-induced inhibition of primary afferent evoked EPSCs is enhanced in complete Freund's adjuvant (CFA)-injected animals. Time plots of the amplitude of dorsal root evoked EPSCs during superfusion of sumatriptan (3 μM) and CNQX (10 μM) in spinal slices from animals which received intraplantar (a) CFA or (b) saline, 3–4 days previously. The insets in (a) and (b) show averaged traces of evoked EPSCs for their respective time plots. (c) Scatter plots (including mean ± SEM) of evoked EPSC amplitude during application of sumatriptan, expressed as a percentage of the pre-agonist value. (d) Scatter plots of the ratio of the amplitude of evoked EPSC2:1 for paired stimuli during application of sumatriptan, expressed as a percentage of the pre-agonist value. Data in (c) and (d) are shown for neurons ipsilateral and contralateral to the hindpaw for animals which received intraplantar CFA, and ipsilateral to the hindpaw for animals which received intraplantar saline ($n = 8, 7, 9$), and * denotes $P < 0.05$ compared to the pre-agonist level; # denotes $P < 0.05$ for post hoc ANOVA comparisons

sumatriptan-induced inhibition of evoked EPSCs, however, significantly varied between the three groups of neurons. The sumatriptan inhibition was significantly greater in ipsilateral and contralateral dorsal horn neurons from complete Freund's adjuvant-injected animals compared to ipsilateral neurons from saline-injected animals (Figure 2c).

3.2 | The sumatriptan-induced inhibition of EPSCs is presynaptic

In the above recordings of evoked EPSCs, we also examined whether the sumatriptan-induced inhibition of synaptic transmission in complete Freund's adjuvant-injected pre-weaned rats was mediated by a presynaptic mechanism. The pre-sumatriptan baseline paired pulse ratio of evoked EPSCs did not differ between ipsilateral and contralateral dorsal horn neurons from complete Freund's adjuvant-injected animals and ipsilateral neurons from saline-injected animals. However, the effect of sumatriptan on the paired pulse ratio significantly varied between the three neuron/treatment groups. Sumatriptan produced a significant increase in the paired pulse ratio of evoked EPSCs in ipsilateral and contralateral dorsal horn neurons from complete Freund's adjuvant-injected animals (Figure 2a,d). By contrast, sumatriptan did not have a significant effect on the paired pulse ratio of evoked EPSCs in ipsilateral neurons from saline-injected animals (Figure 2b,d).

We further examined the role of pre- and postsynaptic mechanisms by examining the effect of sumatriptan on spontaneous miniature EPSCs in ipsilateral dorsal horn neurons from complete Freund's adjuvant-injected pre-weaned animals. In the additional presence of TTX to block action-potential dependent neurotransmitter release, spontaneous miniature EPSCs were observed which were abolished by CNQX (n = 8). Under these conditions, the mean rate but not the amplitude of miniature EPSCs was significantly reduced by sumatriptan (Figure 3a–c). The reduction in miniature EPSC rate was associated with a rightward shift in the cumulative frequency distributions of their inter-event intervals (Figure 3d). Sumatriptan had no effect on the kinetics or the amplitude distributions of miniature EPSCs (Figure 3b,e).

3.3 | Inflammation alters the actions of 5-HT$_1$ subtype selective agonists

To examine which 5-HT$_1$ receptor subtypes mediate the sumatriptan-induced inhibition in pre-weaned rats, we first examined the actions of subtype selective agonists. The effect of the 5-HT$_{1A}$ agonist R (+)-8-OH-DPAT (1 μM) on evoked EPSCs significantly differed between ipsilateral and contralateral neurons from complete Freund's adjuvant-injected animals and ipsilateral neurons from saline-injected animals. R(+)-8-OH-DPAT did not produce a significant change in the amplitude of evoked EPSCs in ipsilateral and contralateral neurons from complete Freund's adjuvant-injected rats (Figure 4a,c). By contrast, R(+)-8-OH-DPAT produced a significant decrease in the amplitude of evoked EPSCs in ipsilateral neurons from saline-injected rats (Figure 4b,c). The effect of R(+)-8-OH-DPAT on the paired pulse ratio

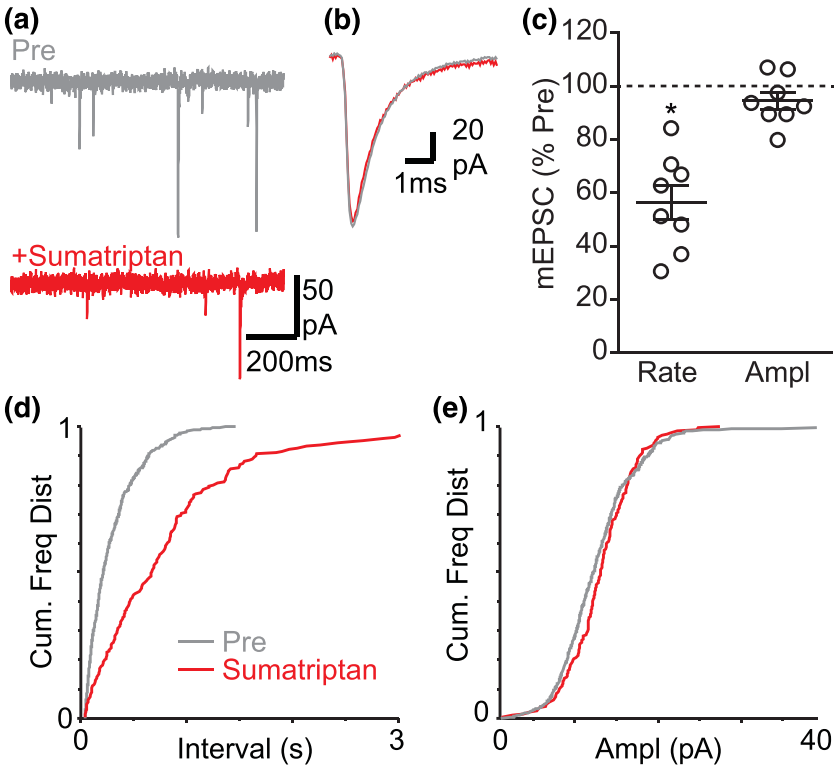

FIGURE 3 Sumatriptan inhibits miniature EPSCs in complete Freund's adjuvant (CFA)-injected animals. (a) Raw traces of spontaneous miniature EPSCs in an ipsilateral dorsal horn neuron from a CFA-injected animal before and during sumatriptan (3 μM). (b) Averaged traces of miniature EPSCs from the neuron in (a). (c) Scatter plots (including mean ± SEM) of miniature EPSC amplitude and rate during application of sumatriptan, expressed as a percentage of the pre-agonist value. Cumulative frequency distribution plots of the (d) inter-event interval and (e) amplitude of miniature EPSCs before and during sumatriptan. In (c) * denotes $P < 0.05$ compared to the pre-agonist level (n = 8)

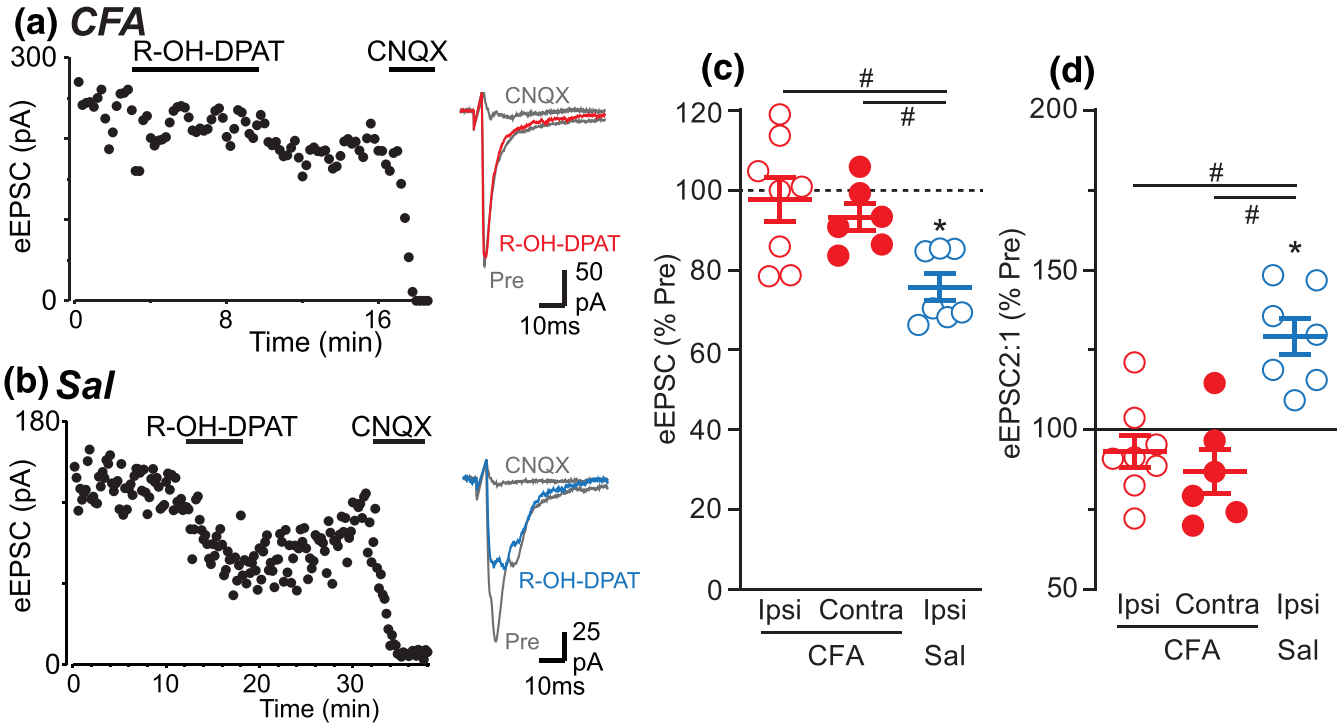

FIGURE 4 R(+)-8-OH-DPAT-induced inhibition of primary afferent evoked EPSCs is reduced in complete Freund's adjuvant (CFA)-injected animals. Time plots of the amplitude of dorsal root evoked EPSCs during superfusion of R(+)-8-OH-DPAT (R-OH-DPAT, 1 μM) and CNQX (10 μM) in spinal slices from animals which received intraplantar (a) CFA or (b) saline, 3–4 days previously. Insets in (a) and (b) show averaged traces of evoked EPSCs taken from epochs in their corresponding time plots. (c) Scatter plots (including mean ± SEM) of evoked EPSC amplitude during application of R(+)-8-OH-DPAT for neurons ipsilateral and contralateral to the hindpaw which received intraplantar CFA, and ipsilateral to the hindpaw which received intraplantar saline (n = 8, 6, 7). (d) Scatter plots of the ratio of the amplitude of evoked EPSC2:1 for the neurons groups described for (c). In (c) and (d) * denotes $P < 0.05$ compared to the pre-agonist level; # denotes $P < 0.05$ for post hoc comparisons

of evoked EPSCs also significantly differed between the three groups of neurons. R(+)-8-OH-DPAT produced an increase in the paired pulse ratio of evoked EPSCs in ipsilateral neurons from saline-injected animals but not in ipsilateral and contralateral neurons from complete Freund's adjuvant-injected animals (Figure 4d).

The effect of the 5-HT_{1B} agonist CP 93129 (1 μM) on evoked EPSCs significantly differed between ipsilateral and contralateral neurons from complete Freund's adjuvant-injected animals and ipsilateral neurons from saline-injected animals. CP 93129 produced a significant decrease the amplitude of evoked EPSCs in ipsilateral and contralateral neurons from complete Freund's adjuvant-injected animals (Figure 5a,c). By contrast, CP 93129 had no effect on the amplitude of evoked EPSCs in neurons ipsilateral to the saline-injected hindpaw. The effect of CP 93129 on the paired pulse ratio of evoked EPSCs also significantly differed between the three groups of neurons. CP 93129 produced a significant increase in the paired pulse ratio of evoked EPSCs in ipsilateral and contralateral neurons from complete Freund's adjuvant-injected animals but not in ipsilateral neurons from saline-injected animals (Figure 5d).

The effect of the 5-HT_{1D} agonist PNU109291 (3 μM) on evoked EPSCs differed between ipsilateral and contralateral neurons from complete Freund's adjuvant-injected animals and ipsilateral neurons from saline-injected animals. PNU109291 produced a significant decrease the amplitude of evoked EPSCs in ipsilateral and contralateral dorsal horn neurons from complete Freund's adjuvant-injected animals (Figure 6a,c). By contrast, PNU109291 did not have a significant effect on evoked EPSC amplitude in ipsilateral neurons from saline-injected animals (Figure 6b,c). The effect of CP 93129 on the paired pulse ratio of evoked EPSCs also significantly differed between the three groups of neurons. PNU109291 produced a significant increase in the paired pulse ratio of evoked EPSCs in ipsilateral and contralateral neurons from complete Freund's adjuvant-injected animals but not in ipsilateral neurons from saline-injected animals (Figure 6d).

3.4 | The inflammation-induced actions of sumatriptan are mediated by 5-HT_{1B} and 5-HT_{1D} receptors

We next examined the effect of subtype selective antagonists on the effect of the selective agonists and sumatriptan in ipsilateral neurons from complete Freund's adjuvant-injected animals. As observed in control untreated slices, R(+)-8-OH-DPAT had no effect on evoked EPSCs in the presence of the 5-HT_{1A} antagonist WAY-100635 (3 μM) (Figure 7a). The effect of CP 93129 on evoked EPSCs was abolished

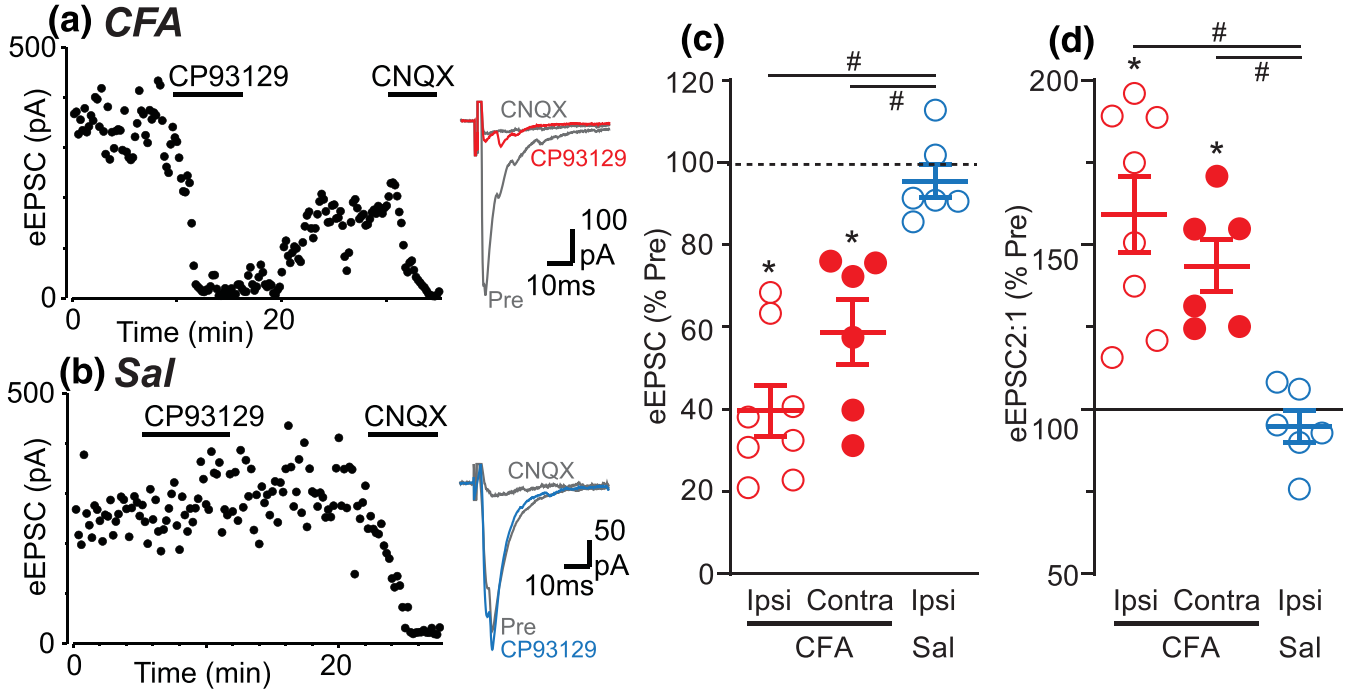

FIGURE 5 CP 93129-induced inhibition of primary afferent evoked EPSCs occurs in complete Freund's adjuvant (CFA) but not saline-injected animals. Time plots of the amplitude of dorsal root evoked EPSCs during superfusion of CP 93129 (1 μM) and CNQX (10 μM) in spinal slices from animals which received intraplantar (a) CFA or (b) saline, 3–4 days previously. Insets in (a) and (b) show averaged traces of evoked EPSCs taken from epochs in corresponding time plots. (c) Scatter plots (including mean ± SEM) of evoked EPSC amplitude during application of CP 93129 for neurons ipsilateral and contralateral to the hindpaw which received intraplantar CFA, and ipsilateral to the hindpaw which received intraplantar saline (n = 8, 6, 6). (d) Scatter plots (including mean ± SEM) of the ratio of the amplitude of evoked EPSC2:1 for the neurons groups described for (c). In (c) and (d) * denotes $P < 0.05$ compared to the pre-agonist level; # denotes $P < 0.05$ for post hoc comparisons

by the 5-HT_{1B} antagonist NAS181 (3 μM) (Figure 7a). In addition, the effect of PNU109291 on evoked EPSCs was abolished by the 5-HT_{1D} antagonist BRL-15572 (3 μM) (Figure 7a). Finally, the effect of sumatriptan on evoked EPSCs significantly varied between neurons from control untreated and antagonist treated slices. Thus, the sumatriptan-induced inhibition of evoked EPSCs was abolished by NAS181 and BRL-15572 but was unaffected by WAY-100635 (Figure 7b).

3.5 | The inflammation-induced enhancement of sumatriptan presynaptic inhibition is developmentally regulated

So far, our data indicate that sumatriptan has a higher efficacy in reducing glutamatergic inputs to dorsal horn neurons in spinal cord slices from pre-weaned rats that have undergone an inflammatory injury. However, there are significant postnatal developmental alterations in spinal pain circuits (Brewer & Baccei, 2019; Fitzgerald, 2015). We therefore examined the actions of sumatriptan in spinal slices from older animals. In P36–56 animals, intraplantar complete Freund's adjuvant produced a significantly greater percentage increase in paw volume compared to saline-injected animals (Figure 1a). The percentage increase in paw volume was not significantly different to that observed in younger P14–21 animals. In these animals, there was a significant decrease in mechanical paw withdrawal threshold of complete Freund's adjuvant but not saline-injected animals (Figure 1b). Thus, intraplanar complete Freund's adjuvant-induced hindpaw inflammation and mechanical allodynia in older animals, as observed in younger pre-weaned animals.

In the P36–56 animals, sumatriptan significantly reduced the amplitude of evoked EPSCs in ipsilateral dorsal horn neurons from both complete Freund's adjuvant- and saline-injected animals (Figure 8a). However, there was no significant difference in the sumatriptan-induced inhibition of evoked EPSCs between the complete Freund's adjuvant- and saline-injected animals (Figure 8b). We then examined the effect of sumatriptan in animals over weekly age epochs, ranging from 1 to 2 to 6–7 weeks of age, to determine the age at which complete Freund's adjuvant-induced enhancement of sumatriptan inhibition ceased. The difference in sumatriptan-induced inhibition in ipsilateral dorsal horn neurons between complete Freund's adjuvant and saline-injected animals significantly varied with age. The sumatriptan-induced inhibition of evoked EPSCs was significantly greater in complete Freund's adjuvant- compared to saline-injected animals which were less than 4 weeks of age (Figure 8b). By contrast, there was no significant difference in the sumatriptan-induced inhibition of evoked EPSCs between complete Freund's

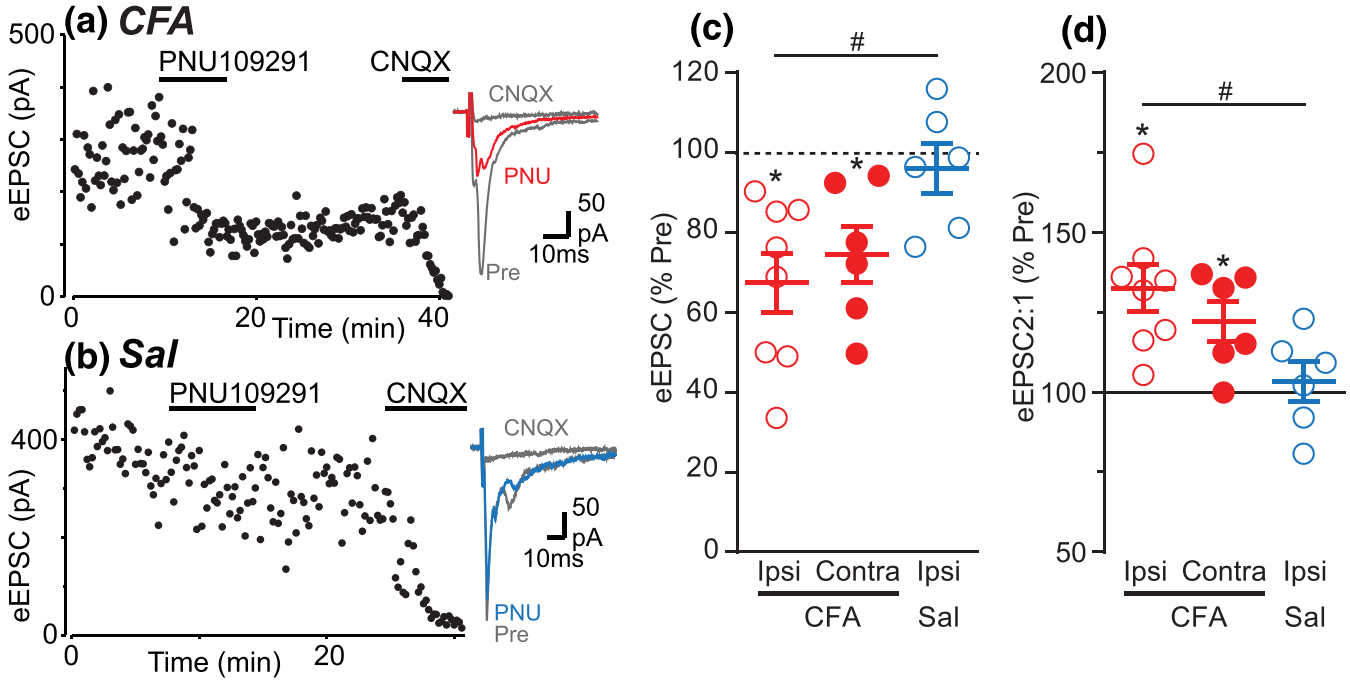

FIGURE 6 PNU109291-induced inhibition of primary afferent evoked EPSCs occurs in complete Freund's adjuvant (CFA) but not saline-injected animals. Time plots of the amplitude of dorsal root evoked EPSCs during superfusion of PNU109291 (3 μM) and CNQX (10 μM) in spinal slices from animals which received intraplantar (a) CFA or (b) saline, 3–4 days previously. Insets in (a) and (b) show averaged traces of evoked EPSCs taken from epochs in corresponding time plots. (c) Scatter plots (including mean ± SEM) of evoked EPSC amplitude during application of PNU109291 for neurons ipsilateral and contralateral to the hindpaw which received intraplantar CFA, and ipsilateral to the hindpaw which received intraplantar saline (n = 8, 6, 6). (d) Scatter plots (including mean ± SEM) of the ratio of the amplitude of evoked EPSC2:1 for the neurons groups described for (c). In (c) and (d) * denotes $P < 0.05$ compared to the pre-agonist level; # denotes $P < 0.05$ for post hoc comparisons

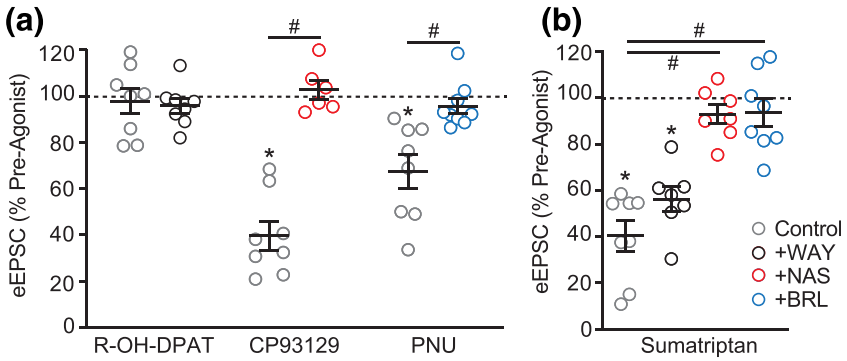

FIGURE 7 The effect of sumatriptan in complete Freund's adjuvant (CFA)-injected animals is mediated by 5-$HT_{1B/1D}$ receptors. (a) Scatter plot of the effect of the 5-HT_1 agonists R(+)-8-OH-DPAT, CP 93129 and PNU109291 (PNU) on evoked EPSC amplitude in untreated slices (control, n = 8, 8, 8), or in slices pre-incubated in the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} antagonists WAY-100635 (WAY, 3 μM, n = 8), NAS181 (NAS 3 μM, n = 6) and BRL-15572 (BRL 3 μM, n = 9), respectively. (b) Scatter plot of the effect of sumatriptan on evoked EPSC amplitude, in untreated (n = 8) and WAY-100635, NAS181 and BRL-15572 treated slices (n = 7, 7, 8). Data are from ipsilateral dorsal horn neurons from CFA-injected animals and is expressed as a percentage of the pre-agonist value. In (a) and (b) * denotes $P < 0.05$ compared to the pre-agonist level; # denotes $P < 0.05$ post hoc comparisons of control untreated versus antagonist treated slices

adjuvant- and saline-injected animals over 4 weeks of age (Figure 8b). It was possible that sex differences confounded this relationship because neurons from both male and female animals were used in all experiments. To assess this, we grouped the two different age range responder types, namely, P8–21 (n = 9, 9 for male and female complete Freund's adjuvant treated; n = 11, 9 for male and female saline-treated) and P28–56 animals (n = 11, 9 for male and female complete Freund's adjuvant treated; n = 8, 8 for male and female saline-treated). The effect of sumatriptan on evoked EPSC amplitude significantly varied with age (groups = P8–21, P28–56) and treatment (groups = complete Freund's adjuvant, saline-injected) but not gender (groups = male, female).

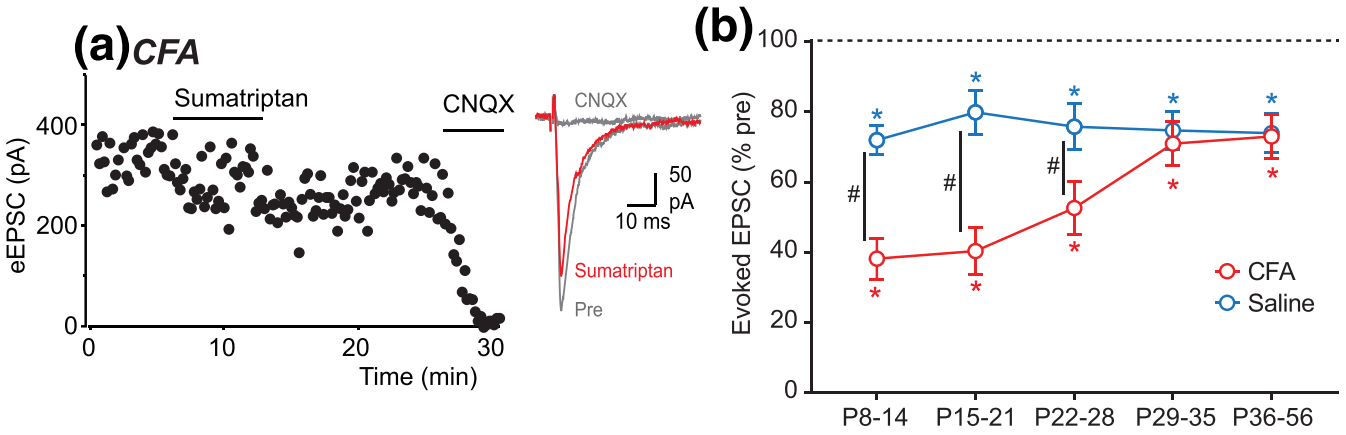

FIGURE 8 The complete Freund's adjuvant (CFA)-induced enhancement of sumatriptan-induced inhibition is age dependent. (a) Time plot of the amplitude of dorsal root evoked EPSCs in a spinal dorsal horn neuron during superfusion of sumatriptan (3 μM) and CNQX (10 μM) from a 42-day-old animal which received intraplantar CFA 3 days previously. The inset shows averaged traces of evoked EPSCs taken from epochs in corresponding time plots. (b) Summary plot of evoked EPSC amplitude during application of sumatriptan for neurons ipsilateral to the hindpaw which received intraplantar CFA or saline (expressed as a percentage of the pre-sumatriptan amplitude), in animals of age P8–14 (n = 10, 11), P15–21 (n = 8, 9), P22–28 (n = 10, 9), P29–35 (n = 10, 7) and P36–56 (n = 10, 9). In (b) * denotes $P < 0.05$ compared to the pre-agonist level; # denotes $P < 0.05$ post hoc comparisons of CFA versus saline-treated animals

4 | DISCUSSION

In the present study, it has been demonstrated that the inhibition of primary afferent synaptic transmission into the lumbar superficial dorsal horn by sumatriptan is enhanced by the complete Freund's adjuvant-induced model of inflammatory pain. The inflammation-induced enhancement was mediated by an increase in presynaptic 5-$HT_{1B/1D}$ receptor inhibition. However, this inflammation-induced enhancement was developmentally regulated and ceased by the onset of peri-adolescence. These findings provide a cellular basis for sumatriptan-induced spinal analgesia in a juvenile setting.

4.1 | Inflammation enhances sumatriptan presynaptic inhibition of primary afferent synaptic transmission

In the present study, it was found the triptan, sumatriptan, inhibited afferent evoked, non-NMDA mediated EPSCs onto superficial dorsal horn neurons from juvenile 2- to 3-week-old rats which had previously received intraplantar injections of complete Freund's adjuvant or saline. A number of observations indicated that this inhibition were due to a presynaptic reduction in glutamate release from primary afferent inputs. First, the sumatriptan-induced inhibition of afferent evoked EPSCs was associated with an increase in their paired pulse ratio, as observed previously for other serotonergic agents in the spinal and trigeminal dorsal horn (Hori et al., 1996; Jeong et al., 2012; Travagli & Williams, 1996). Second, sumatriptan produced a reduction in the rate but not the amplitude of spontaneous miniature EPSCs in dorsal horn neurons from complete Freund's adjuvant-injected rats. Finally, sumatriptan had no effect on membrane conductance, as observed in other studies on medullary dorsal horn (Choi et al., 2012; Jennings et al., 2004; Travagli & Williams, 1996). These observations were also similar to the presynaptic effects of sumatriptan and 5-$HT_{1B/1D}$ agonists on synaptic transmission previously observed within a number of brain regions (Best & Regehr, 2008; Guo & Rainnie, 2010; Hwang & Chung, 2014; Mathur, Capik, Alvarez, & Lovinger, 2011; Mizutani, Hori, & Takahashi, 2006).

The sumatriptan-induced inhibition was greater in animals which had previously received intraplantar complete Freund's adjuvant-injection compared to those which received intraplantar saline. This difference was likely to be specifically related to the effects of complete Freund's adjuvant because the lesser inhibition induced by sumatriptan in saline-injected animals (22 ± 9% inhibition) was similar to that observed in our prior study on spinal slices from naïve, unoperated animals (28 ± 5% inhibition) of a similar age (Jeong et al., 2012). Interestingly, it was also observed that the sumatriptan-induced inhibition of evoked EPSCs in complete Freund's adjuvant- but not saline-injected animals did not always reverse upon washout of sumatriptan. In this regard, it has been observed that sumatriptan and other 5-$HT_{1B/1D}$ agonists produce long-term depression of synaptic transmission in brain regions such as the habenula and nucleus accumbens (Hwang & Chung, 2014). These findings indicate that sumatriptan-induced presynaptic inhibition of afferent inputs onto spinal dorsal horn neurons is enhanced by complete Freund's adjuvant inflammation.

4.2 | Inflammation alters the contribution of presynaptic 5-HT_1 receptor subtypes

While sumatriptan is used as an agonist at 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1F} receptors (Humphrey et al., 1990), it also has affinity for 5-HT_{1A} receptors (Newman-Tancredi et al., 1997; Schoeffter &

Hoyer, 1989). In the present study, complete Freund's adjuvant-induced inflammation led to differential changes in 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptor mediated effects on synaptic transmission in dorsal horn neurons of juvenile animals. It was observed that the 5-HT_{1B} and 5-HT_{1D} agonists CP 93129 and PNU109291 inhibited evoked EPSCs in dorsal horn neurons from complete Freund's adjuvant but not saline-injected animals and this inhibition was abolished by 5-HT_{1B} and 5-HT_{1D} selective antagonists, NAS181 and BRL-15572. By contrast, the 5-HT_{1A} agonist R(+)-8-OH-DPAT inhibited evoked EPSCs in dorsal horn neurons from saline- but not complete Freund's adjuvant-injected animals. These observations suggest that complete Freund's adjuvant-induced inflammation leads to an up-regulation of presynaptic 5-HT_{1B} and 5-HT_{1D} receptor mediated inhibition but a down-regulation of presynaptic 5-HT_{1A} receptor inhibition in spinal pain circuits. While the effect of inflammation on postsynaptic actions was not examined in the present study, it should be noted that 5-HT_{1A} but not 5-HT_{1B} receptor activation produces postsynaptic inhibition of spinal/medullary dorsal horn neurons from naïve animals (Abe et al., 2009; Choi et al., 2012; Jennings et al., 2004; Travagli & Williams, 1996).

In the present study, the sumatriptan-induced inhibition of evoked EPSCs in complete Freund's adjuvant-injected animals was abolished by NAS181 and BRL-15572 but was unaffected by the 5-HT_{1A} antagonist WAY-100635. This indicates that the presynaptic actions of sumatriptan in complete Freund's adjuvant-injected animals were mediated by 5-HT_{1B} and 5-HT_{1D} receptors. This differs to our prior study on spinal dorsal horn slices from naïve, unoperated animals where the sumatriptan-induced inhibition is exclusively mediated by 5-HT1A receptors (Jeong et al., 2012). Interestingly, however, the current observations parallel those in the trigeminal dorsal horn of naïve rats where both 5-HT_{1B} and 5-HT_{1D} receptors have a role in sumatriptan-induced inhibition of glutamatergic synaptic transmission (Choi et al., 2012; Jennings et al., 2004). It might be noted that there appears to be some redundancy in the contribution of spinal 5-HT_{1B} and 5-HT_{1D} receptors because antagonists for both receptor subtypes individually abolished the sumatriptan-induced inhibition.

There are a number of potential reasons why spinal 5-$HT_{1B/1D}$ presynaptic inhibition was observed in an inflammatory pain model but not in control and naïve animals. Like the trigeminal system, 5-HT_{1B} and 5-HT_{1D} receptor mRNA and protein is present within lumbar dorsal root ganglion neurons (Classey et al., 2010; Pierce et al., 1996; Potrebic et al., 2003; Wotherspoon & Priestley, 2000). It has been shown that inflammation leads to an increase in 5-HT_{1B} and 5-HT_{1D} mRNA in dorsal root ganglia, although other studies suggest a more complex time dependence (Ahn & Basbaum, 2006; Liu et al., 2005; Wu et al., 2001). In addition to this, 5-HT_{1D} receptors are located within dense core vesicles in naïve animals, rather than on the plasma membrane (Ahn & Basbaum, 2006; Potrebic et al., 2003) and inflammation increases membrane expression of 5-HT_{1D} receptors within the dorsal horn (Ahn & Basbaum, 2006). It is therefore possible that inflammation leads to increased trafficking of 5-$HT_{1B/1D}$ receptors to the membrane making them accessible to exogenously applied triptans, as has been observed for delta-opioid receptors (Morinville, Cahill, Kieffer, Collier, & Beaudet, 2004). Another potential mechanism is related to inflammation induce adaptations in the presynaptic release machinery. The lack of difference in the paired pulse ratio of baseline evoked EPSCs between saline and complete Freund's adjuvant treated animals indicated that inflammation did not alter the probability of release from presynaptic terminals, as observed previously (Rycroft, Vikman, & Christie, 2007). However, inflammation alters the subtypes of presynaptic voltage gated calcium channels involved in transmitter release (Rycroft et al., 2007), and these may differentially modulated by 5-HT_1 receptor subtypes (Lin, Setya, Johnson-Farley, & Cowen, 2002). However, it remains to be determined whether these account for the inflammation-induced enhancement of sumatriptan-induced inhibition observed in the present study.

Interestingly, the enhancement of the 5-$HT_{1B/1D}$ inhibition of evoked EPSCs was also observed in dorsal horn neurons contralateral to the site of complete Freund's adjuvant-injection. While the precise mechanisms underlying these contralateral changes remain to be determined, it has previously been shown that inflammation produces bilateral adaptations in the spinal dorsal horn which are mediated by microglial activation (Cahill, Morinville, Hoffert, O'Donnell, & Beaudet, 2003; Choi et al., 2015; Schreiber, Beitz, & Wilcox, 2008). Furthermore, it has been shown that complete Freund's adjuvant induces a bilateral increase in 5-HT_{1B} mRNA expression in dorsal root ganglion neurons (Wu et al., 2001). Thus, the observed contralateral effects could be due to adaptation in primary afferent terminals within the dorsal horn.

4.3 | The inflammation-induced enhancement of sumatriptan inhibition is age dependent

The other major finding of the present study was that the sumatriptan inhibition of glutamatergic synaptic transmission into the spinal dorsal horn was only enhanced by complete Freund's adjuvant in rats up to an age of 3–4 weeks. This age equates to the onset of peri-adolescence, which is prior to the development of reproductive maturity and is an age at which there are pronounced behavioural and pharmacological changes (Spear & Brake, 1983). The neurochemical, neuroanatomical and behavioural changes that occur during this period in rats are similar to those seen in human adolescents (Tirelli, Laviola, & Adriani, 2003). This suggests that the inflammation-induced enhancement of spinal sumatriptan inhibition is restricted to pre-adolescent rats. While their developmental expression in primary afferents has not been examined previously, it has been shown that 5-$HT_{1B/1D}$ levels within the rat and human brain, plus 5-HT_{1B} levels within the rat spinal cord are transiently enhanced during the pre-adolescent period (del Olmo et al., 1996; Liu & Wong-Riley, 2010; Pranzatelli & Galvan, 1994).

It has previously been demonstrated that spinally delivered sumatriptan reduces inflammation-induced pain in adult mice (Bingham et al., 2001; Nikai et al., 2008). While not systematically examined, it might be noted that the spinal antinociceptive effect of epicatechin in the carrageenan model of inflammation in adult rats is reduced by

5-$HT_{1B/1D}$ antagonists and also by 5-HT_{1A} and opioid antagonists (Quinonez-Bastidas et al., 2018). Furthermore, spinally delivered sumatriptan and 5-$HT_{1B/1D}$ agonists lack antinociceptive activity in naïve adult rats (Connor et al., 1997; Jeong et al., 2012; Skingle et al., 1990). It therefore remains to be determined whether sumatriptan reduces inflammatory pain in rats and whether this is developmentally regulated.

5 | CONCLUSIONS

The present findings suggest that, at the spinal level, inflammation induces presynaptic 5-$HT_{1B/1D}$ receptor mediated inhibition of transmitter release from presynaptic terminals of primary afferents within the spinal dorsal horn. This novel expression of 5-$HT_{1B/1D}$ presynaptic inhibition is developmentally regulated and restricted to pre-adolescent animals. These findings indicate triptans might have analgesic potential in a juvenile setting.

ACKNOWLEDGEMENT

This study was funded by NHMRC Grant 1083569.

AUTHOR CONTRIBUTIONS

C.W.V. devised the project. All authors performed and analysed the experiments and wrote and approved the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the *BJP* guidelines for Design & Analysis, and Animal Experimentation, and as recommended by funding agencies, publishers and other organisations engaged with supporting research.

ORCID

Bryony L. Winters https://orcid.org/0000-0002-4973-0771

Christopher W. Vaughan https://orcid.org/0000-0003-4314-7689

REFERENCES

Abe, K., Kato, G., Katafuchi, T., Tamae, A., Furue, H., & Yoshimura, M. (2009). Responses to 5-HT in morphologically identified neurons in the rat substantia gelatinosa in vitro. *Neuroscience*, *159*, 316–324. https://doi.org/10.1016/j.neuroscience.2008.12.021

Ahn, A. H., & Basbaum, A. I. (2006). Tissue injury regulates serotonin 1D receptor expression: Implications for the control of migraine and inflammatory pain. *The Journal of Neuroscience*, *26*, 8332–8338. https://doi.org/10.1523/JNEUROSCI.1989-06.2006

Akerman, S., Holland, P. R., Summ, O., Lasalandra, M. P., & Goadsby, P. J. (2012). A translational in vivo model of trigeminal autonomic cephalalgias: Therapeutic characterization. *Brain*, *135*, 3664–3675. https://doi.org/10.1093/brain/aws249

Akerman, S., Karsan, N., Bose, P., Hoffmann, J. R., Holland, P. R., Romero-Reyes, M., & Goadsby, P. J. (2019). Nitroglycerine triggers triptan-responsive cranial allodynia and trigeminal neuronal hypersensitivity. *Brain*, *142*, 103–119. https://doi.org/10.1093/brain/awy313

Alexander, S. P. H., Christopoulos, A., Davenport, A. P., Kelly, E., Mathie, A., Peters, J. A., … Pawson, A. J. (2019). The concise guide to pharmacology 2019/20: G protein-coupled receptors. *Brit J Pharmacol*, *176*(Suppl 1), S21–S141.

Alexander, S. P. H., Mathie, A., Peters, J. A., Veale, E. L., Striessnig, J., Kelly, E., … Sharman, J. L. (2019). The concise guide to pharmacology 2019/20: Ion channels. *Brit J Pharmacol*, *176*(Suppl 1), S142–S228.

Alhaider, A. A., & Wilcox, G. L. (1993). Differential roles of 5-hydroxytryptamine1A and 5-hydroxytryptamine1B receptor subtypes in modulating spinal nociceptive transmission in mice. *The Journal of Pharmacology and Experimental Therapeutics*, *265*, 378–385.

Ali, Z., Wu, G., Kozlov, A., & Barasi, S. (1994). The actions of 5-HT1 agonists and antagonists on nociceptive processing in the rat spinal cord: Results from behavioural and electrophysiological studies. *Brain Research*, *661*, 83–90. https://doi.org/10.1016/0006-8993(94)91184-3

Best, A. R., & Regehr, W. G. (2008). Serotonin evokes endocannabinoid release and retrogradely suppresses excitatory synapses. *The Journal of Neuroscience*, *28*, 6508–6515. https://doi.org/10.1523/JNEUROSCI.0678-08.2008

Bingham, S., Davey, P. T., Sammons, M., Raval, P., Overend, P., & Parsons, A. A. (2001). Inhibition of inflammation-induced thermal hypersensitivity by sumatriptan through activation of 5-HT(1B/1D) receptors. *Experimental Neurology*, *167*, 65–73. https://doi.org/10.1006/exnr.2000.7521

Bonin, R. P., Bories, C., & De Koninck, Y. (2014). A simplified up-down method (SUDO) for measuring mechanical nociception in rodents using von Frey filaments. *Molecular Pain*, *10*, 26.

Brewer, C. L., & Baccei, M. L. (2019). The development of pain circuits and unique effects of neonatal injury. *Journal of Neural Transmission (Vienna).127*(4), 467–479.

Cahill, C. M., Morinville, A., Hoffert, C., O'Donnell, D., & Beaudet, A. (2003). Up-regulation and trafficking of delta opioid receptor in a model of chronic inflammation: Implications for pain control. *Pain*, *101*, 199–208. https://doi.org/10.1016/s0304-3959(02)00333-0

Cameron, C., Kelly, S., Hsieh, S. C., Murphy, M., Chen, L., Kotb, A., … Wells, G. (2015). Triptans in the acute treatment of migraine: A systematic review and network meta-analysis. *Headache*, *55*(Suppl 4), 221–235. https://doi.org/10.1111/head.12601

Choi, H. S., Roh, D. H., Yoon, S. Y., Moon, J. Y., Choi, S. R., Kwon, S. G., … Lee, J. H. (2015). Microglial interleukin-1β in the ipsilateral dorsal horn inhibits the development of mirror-image contralateral mechanical allodynia through astrocyte activation in a rat model of inflammatory pain. *Pain*, *156*, 1046–1059. https://doi.org/10.1097/j.pain.0000000000000148

Choi, I. S., Cho, J. H., An, C. H., Jung, J. K., Hur, Y. K., Choi, J. K., & Jang, I. S. (2012). 5-HT(1B) receptors inhibit glutamate release from primary afferent terminals in rat medullary dorsal horn neurons. *Brit J Pharmacol*, *167*, 356–367. https://doi.org/10.1111/j.1476-5381.2012.01964.x

Classey, J. D., Bartsch, T., & Goadsby, P. J. (2010). Distribution of 5-HT (1B), 5-HT(1D) and 5-HT(1F) receptor expression in rat trigeminal and dorsal root ganglia neurons: Relevance to the selective anti-migraine effect of triptans. *Brain Research*, *1361*, 76–85. https://doi.org/10.1016/j.brainres.2010.09.004

Connor, H. E., Feniuk, W., Beattie, D. T., North, P. C., Oxford, A. W., Saynor, D. A., & Humphrey, P. P. (1997). Naratriptan: Biological profile in animal models relevant to migraine. *Cephalalgia*, *17*, 145–152. https://doi.org/10.1046/j.1468-2982.1997.1703145.x

Cumberbatch, M. J., Hill, R. G., & Hargreaves, R. J. (1998). Differential effects of the 5HT1B/1D receptor agonist naratriptan on trigeminal

versus spinal nociceptive responses. *Cephalalgia*, *18*, 659–663. https://doi.org/10.1046/j.1468-2982.1998.1810659.x

Curtis, M. J., Alexander, S., Cirino, G., Docherty, J. R., George, C. H., Giembycz, M. A., … Ahluwalia, A. (2018). Experimental design and analysis and their reporting II: Updated and simplified guidance for authors and peer reviewers. *Brit J Pharmacol*, *175*, 987–993. https://doi.org/10.1111/bph.14153

del Olmo, E., del Arco, C., Diaz, A., Pascual, J., Mengod, G., Palacios, J. M., & Pazos, A. (1996). Ontogenetic development of 5-HT1D receptors in human brain: An autoradiographic study. *The European Journal of Neuroscience*, *8*, 53–60. https://doi.org/10.1111/j.1460-9568.1996.tb01166.x

Donaldson, C., Boers, P. M., Hoskin, K. L., Zagami, A. S., & Lambert, G. A. (2002). The role of 5-HT1B and 5-HT1D receptors in the selective inhibitory effect of naratriptan on trigeminovascular neurons. *Neuropharmacology*, *42*, 374–385. https://doi.org/10.1016/s0028-3908(01)00190-3

Fitzgerald, M. (2015). What do we really know about newborn infant pain? *Experimental Physiology*, *100*, 1451–1457.

Garraway, S. M., & Hochman, S. (2001). Pharmacological characterization of serotonin receptor subtypes modulating primary afferent input to deep dorsal horn neurons in the neonatal rat. *Brit J Pharmacol*, *132*, 1789–1798.

Goadsby, P. J., & Hoskin, K. L. (1996). Inhibition of trigeminal neurons by intravenous administration of the serotonin (5HT)(1B/D) receptor agonist zolmitriptan (311C90): Are brain stem sites therapeutic target in migraine? *Pain*, *67*, 355–359. https://doi.org/10.1016/0304-3959(96)03118-1

Guo, J. D., & Rainnie, D. G. (2010). Presynaptic 5-HT(1B) receptor-mediated serotonergic inhibition of glutamate transmission in the bed nucleus of the stria terminalis. *Neuroscience*, *165*, 1390–1401. https://doi.org/10.1016/j.neuroscience.2009.11.071

Harding, S. D., Sharman, J. L., Faccenda, E., Southan, C., Pawson, A. J., Ireland, S., … NC-IUPHAR. (2018). The IUPHAR/BPS guide to pharmacology in 2018: Updates and expansion to encompass the new guide to immunopharmacology. *Nucleic Acids Research*, *46*, D1091–D1106. https://doi.org/10.1093/nar/gkx1121

Hori, Y., Endo, K., & Takahashi, T. (1996). Long-lasting synaptic facilitation induced by serotonin in superficial dorsal horn neurones of the rat spinal cord. *Journal of Physiology (London)*, *492*, 867–876.

Hoskin, K. L., & Goadsby, P. J. (1998). Comparison of more and less lipophilic serotonin (5HT1B/1D) agonists in a model of trigeminovascular nociception in cat. *Experimental Neurology*, *150*, 45–51. https://doi.org/10.1006/exnr.1997.6749

Humphrey, P. P. A., Feniuk, W., Perren, M. J., Beresford, I. J., Skingle, M., & Whalley, E. T. (1990). Serotonin and migraine. *Annals of the new York Academy of Sciences*, *600*, 587–598.

Hwang, E. K., & Chung, J. M. (2014). 5HT(1B) receptor-mediated presynaptic depression of excitatory inputs to the rat lateral habenula. *Neuropharmacology*, *81*, 153–165. https://doi.org/10.1016/j.neuropharm.2014.01.046

Ito, A., Kumamoto, E., Takeda, M., Shibata, K., Sagai, H., & Yoshimura, M. (2000). Mechanisms for ovariectomy-induced hyperalgesia and its relief by calcitonin: Participation of 5-HT1A-like receptor on C-afferent terminals in substantia gelatinosa of the rat spinal cord. *The Journal of Neuroscience*, *20*, 6302–6308.

Jennings, E. A., Ryan, R. M., & Christie, M. J. (2004). Effects of sumatriptan on rat medullary dorsal horn neurons. *Pain*, *111*, 30–37. https://doi.org/10.1016/j.pain.2004.05.018

Jeong, H. J., Mitchell, V. A., & Vaughan, C. W. (2012). Role of 5-HT (1) receptor subtypes in the modulation of pain and synaptic transmission in rat spinal superficial dorsal horn. *Brit J Pharmacol*, *165*, 1956–1965.

Kayser, V., Aubel, B., Hamon, M., & Bourgoin, S. (2002). The antimigraine 5-HT 1B/1D receptor agonists, sumatriptan, zolmitriptan and dihydroergotamine, attenuate pain-related behaviour in a rat model of trigeminal neuropathic pain. *Brit J Pharmacol*, *137*, 1287–1297.

Kilkenny, C., Browne, W., Cuthill, I. C., Emerson, M., & Altman, D. G. (2010). Animal research: Reporting in vivo experiments: The ARRIVE guidelines. *British Journal of Pharmacology*, *160*, 1577–1579.

Levy, D., Jakubowski, M., & Burstein, R. (2004). Disruption of communication between peripheral and central trigeminovascular neurons mediates the antimigraine action of 5HT 1B/1D receptor agonists. *Proceedings of the National Academy of Sciences of the United States of America*, *101*, 4274–4279. https://doi.org/10.1073/pnas.0306147101

Li, P., & Zhuo, M. (1998). Silent glutamatergic synapses and nociception in mammalian spinal cord. *Nature*, *393*, 695–698. https://doi.org/10.1038/31496

Lin, S. L., Setya, S., Johnson-Farley, N. N., & Cowen, D. S. (2002). Differential coupling of 5-HT(1) receptors to G proteins of the G(i) family. *Brit J Pharmacol*, *136*, 1072–1078.

Liu, Q., & Wong-Riley, M. T. (2010). Postnatal changes in the expressions of serotonin 1A, 1B, and 2A receptors in ten brain stem nuclei of the rat: Implication for a sensitive period. *Neuroscience*, *165*, 61–78. https://doi.org/10.1016/j.neuroscience.2009.09.078

Liu, X. Y., Wu, S. X., Wang, Y. Y., Wang, W., Zhou, L., & Li, Y. Q. (2005). Changes of 5-HT receptor subtype mRNAs in rat dorsal root ganglion by bee venom-induced inflammatory pain. *Neuroscience Letters*, *375*, 42–46. https://doi.org/10.1016/j.neulet.2004.10.064

Lu, Y., & Perl, E. R. (2007). Selective action of noradrenaline and serotonin on neurones of the spinal superficial dorsal horn in the rat. *Journal of Physiology (London)*, *582*, 127–136.

Mathur, B. N., Capik, N. A., Alvarez, V. A., & Lovinger, D. M. (2011). Serotonin induces long-term depression at corticostriatal synapses. *The Journal of Neuroscience*, *31*, 7402–7411. https://doi.org/10.1523/JNEUROSCI.6250-10.2011

Mizutani, H., Hori, T., & Takahashi, T. (2006). 5-HT1B receptor-mediated presynaptic inhibition at the calyx of Held of immature rats. *The European Journal of Neuroscience*, *24*, 1946–1954. https://doi.org/10.1111/j.1460-9568.2006.05063.x

Monteith, T. S., & Goadsby, P. J. (2011). Acute migraine therapy: New drugs and new approaches. *Current Treatment Options in Neurology*, *13*, 1–14. https://doi.org/10.1007/s11940-010-0105-6

Morinville, A., Cahill, C. M., Kieffer, B., Collier, B., & Beaudet, A. (2004). Mu-opioid receptor knockout prevents changes in delta-opioid receptor trafficking induced by chronic inflammatory pain. *Pain*, *109*, 266–273.

Newman-Tancredi, A., Conte, C., Chaput, C., Verriele, L., Audinot-Bouchez, V., Lochon, S., … Millan, M. J. (1997). Agonist activity of antimigraine drugs at recombinant human 5-HT1A receptors: Potential implications for prophylactic and acute therapy. *N-S Arch Pharmacol*, *355*, 682–688. https://doi.org/10.1007/PL00005000

Nikai, T., Basbaum, A. I., & Ahn, A. H. (2008). Profound reduction of somatic and visceral pain in mice by intrathecal administration of the anti-migraine drug, sumatriptan. *Pain*, *139*, 533–540. https://doi.org/10.1016/j.pain.2008.06.002

Nozaki, K., Moskowitz, M. A., & Boccalini, P. (1992). CP-93,129, sumatriptan, dihydroergotamine block c-fos expression within rat trigeminal nucleus caudalis caused by chemical stimulation of the meninges. *Brit J Pharmacol*, *106*, 409–415.

Pierce, P. A., Xie, G. X., Levine, J. D., & Peroutka, S. J. (1996). 5-Hydroxytryptamine receptor subtype messenger RNAs in rat peripheral sensory and sympathetic ganglia: A polymerase chain reaction study. *Neuroscience*, *70*, 553–559. https://doi.org/10.1016/0306-4522(95)00329-0

Potrebic, S., Ahn, A. H., Skinner, K., Fields, H. L., & Basbaum, A. I. (2003). Peptidergic nociceptors of both trigeminal and dorsal root ganglia express serotonin 1D receptors: Implications for the selective antimigraine action of triptans. *The Journal of Neuroscience*, *23*, 10988–10997.

Pranzatelli, M. R., & Galvan, I. (1994). Ontogeny of [125I] iodocyanopindolol-labelled 5-hydroxytryptamine1B-binding sites in the rat CNS. *Neuroscience Letters*, *167*, 166–170. https://doi.org/10.1016/0304-3940(94)91053-7

Quinonez-Bastidas, G. N., Pineda-Farias, J. B., Flores-Murrieta, F. J., Rodriguez-Silverio, J., Reyes-Garcia, J. G., Godinez-Chaparro, B., … Rocha-González, H. I. (2018). Antinociceptive effect of (–)-epicatechin in inflammatory and neuropathic pain in rats. *Behavioural Pharmacology*, *29*, 270–279. https://doi.org/10.1097/FBP.0000000000000320

Rycroft, B. K., Vikman, K. S., & Christie, M. J. (2007). Inflammation reduces the contribution of N-type calcium channels to primary afferent synaptic transmission onto NK1 receptor-positive lamina I neurons in the rat dorsal horn. *Journal of Physiology (London)*, *580*, 883–894.

Schoeffter, P., & Hoyer, D. (1989). How selective is GR 43175? Interactions with functional 5-HT1A, 5-HT1B, 5-HT1C and 5-HT1D receptors. *N-S Archieves of Pharmacology*, *340*, 135–138.

Schreiber, K. L., Beitz, A. J., & Wilcox, G. L. (2008). Activation of spinal microglia in a murine model of peripheral inflammation-induced, long-lasting contralateral allodynia. *Neuroscience Letters*, *440*, 63–67. https://doi.org/10.1016/j.neulet.2008.05.044

Skingle, M., Birch, P. J., Leighton, G. E., & Humphrey, P. P. (1990). Lack of antinociceptive activity of sumatriptan in rodents. *Cephalalgia*, *10*, 207–212. https://doi.org/10.1046/j.1468-2982.1990.1005207.x

Spear, L. P., & Brake, S. C. (1983). Periadolescence: Age-dependent behavior and psychopharmacological responsivity in rats. *Developmental Psychobiology*, *16*, 83–109. https://doi.org/10.1002/dev.420160203

Storer, R. J., & Goadsby, P. J. (1997). Microiontophoretic application of serotonin (5HT)1B/1D agonists inhibits trigeminal cell firing in the cat. *Brain*, *120*, 2171–2177.

Ting, J. T., Daigle, T. L., Chen, Q., & Feng, G. (2014). Acute brain slice methods for adult and aging animals: Application of targeted patch clamp analysis and optogenetics. *Methods in Molecular Biology*, *1183*, 221–242. https://doi.org/10.1007/978-1-4939-1096-0_14

Tirelli, E., Laviola, G., & Adriani, W. (2003). Ontogenesis of behavioral sensitization and conditioned place preference induced by psychostimulants in laboratory rodents. *Neuroscience and Biobehavioral Reviews*, *27*, 163–178. https://doi.org/10.112016/s0149-7634(03)00018-6

Travagli, R. A., & Williams, J. T. (1996). Endogenous monoamines inhibit glutamate transmission in the spinal trigeminal nucleus of the guinea-pig. *Journal of Physiology (London)*, *491*, 177–185.

Walker, S. M., Meredith-Middleton, J., Cooke-Yarborough, C., & Fitzgerald, M. (2003). Neonatal inflammation and primary afferent terminal plasticity in the rat dorsal horn. *Pain*, *105*, 185–195. https://doi.org/10.1016/s0304-3959(03)00201-x

Wotherspoon, G., & Priestley, J. V. (2000). Expression of the 5-HT1B receptor by subtypes of rat trigeminal ganglion cells. *Neuroscience*, *95*, 465–471.

Wu, S., Zhu, M., Wang, W., Wang, Y., Li, Y., & Yew, D. T. (2001). Changes of the expression of 5-HT receptor subtype mRNAs in rat dorsal root ganglion by complete Freund's adjuvant-induced inflammation. *Neuroscience Letters*, *307*, 183–186. https://doi.org/10.1016/s0304-3940(01)01946-2

Xu, W., Qiu, X. C., & Han, J. S. (1994). Serotonin receptor subtypes in spinal antinociception in the rat. *The Journal of Pharmacology and Experimental Therapeutics*, *269*, 1182–1189.

Zhang, Y. Q., Gao, X., Ji, G. C., Huang, Y. L., Wu, G. C., & Zhao, Z. Q. (2002). Expression of 5-HT1A receptor mRNA in rat lumbar spinal dorsal horn neurons after peripheral inflammation. *Pain*, *98*, 287–295. https://doi.org/10.1016/s0304-3959(02)00026-x

How to cite this article: Winters BL, Jeong H-J, Vaughan CW. Inflammation induces developmentally regulated sumatriptan inhibition of spinal synaptic transmission. *Br J Pharmacol*. 2020;177:3730–3743. https://doi.org/10.1111/bph.15089